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THE UNIVERSITY OF ALBERTA

THE EFFECT OF TOTAL PARENTERAL NUTRITION ON BILIARY LIPIDS IN NEONATES

BY

(C)

Abdollah A. M. ALrabeeah

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE

OF MASTER OF SCIENCE

IN

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DEPARTMENT OF SURGERY

EDMONTON, ALBERTA

SPRING, 1986

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(Signed). alrabeeah

P.O. Box 54385

Riyadh 11514

Saudi Arabia

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FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled The Effect of Total Parenteral Nutrition on Biliary Lipids in Neonates submitted by Abdollah A.M. ALrabeeah in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE IN EXPERIMENTAL SURGERY.

Olin C. Thurston
.....
(Supervisor)

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April 17, 1986

TO MY PARENTS, WIFE AND SON

ABSTRACT

Total parenteral nutrition is given to patients in a neonatal intensive care unit in a milieu that includes prematurity, critical illness and prolonged absence of oral intake. Disturbances of biliary physiology are common in these patients and are frequently attributed to total parenteral nutrition. In this study we have examined biliary lipids (total bile acids, phospholipid and cholesterol) in neonates in an intensive care unit in an attempt to separate the effects of total parenteral nutrition from those of prolonged fasting on biliary lipids. Also the gallbladder was examined by ultrasound on neonates on prolonged parenteral feeding.

Duodenal bile for lipid analysis was obtained by intubation from 13 neonates (average weight = 1938 g) fasting 3 - 6 days prior to starting total parenteral feeding and from 17 neonates (average weight 2378 g) receiving total parenteral nutrition for up to 49 days as their only nutrition.

Biliary cholesterol rose abruptly from unsaturated levels to supersaturated levels during six days fast prior to initiation of total parenteral nutrition ($y = -18.7 + 7.4x$, $r = 0.90$). Mean cholesterol as a percent of total biliary lipids for this group was 14.2 ∓ 2.0 (SEM). The introduction of total parenteral nutrition appeared to have an early beneficial effect on biliary lipid composition as percent cholesterol fell to 10.4 ∓ 1.9 in patients tested at 14 days but later rose to 18.7 ∓ 1.9 percent in patients studied after 21 - 49 days of total parenteral therapy ($p < 0.01$)

indicating bile extremely supersaturated with cholesterol. Also the total biliary lipid concentration was affected by fasting and parenteral nutrition. It appeared that the total biliary lipid concentration decreased in the fasting neonates and those on prolonged total parenteral nutrition. However the initiation of total parenteral nutrition resulted in a rise in biliary lipid concentration during the first 14 days ($p < 0.01$).

The results of this study suggest that the neonatal liver is unable to produce bile capable of solubilizing cholesterol beyond 3 days fasting. This situation is comparable qualitatively to the adult situation but the degree of cholesterol supersaturation seen at 5 - 6 days of fasting is beyond that seen in adults. The introduction of total parenteral nutrition during the fasting state actually lowers the percentage of cholesterol in bile and increases the total biliary lipid concentration for about 2 weeks but beyond that time the percentage of cholesterol in bile rises again and the total biliary lipid concentration drops to levels seen in the fasting neonates.

In conclusion, it is possible to state that prolonged fasting increases biliary cholesterol saturation and decreases total biliary lipid concentration in neonates and that total parenteral nutrition has a beneficial effect on biliary lipid composition and concentration during the first 14 days.

PREFACE

The energy reserve of the preterm infant is limited whereas the energy requirements are high, especially in the sick infant. Therefore, in the sick preterm infant with poor gastrointestinal function, energy must be supplied parenterally in order to achieve normal growth and development. However, the use of total parenteral nutrition is associated with complications. One of the many complications that have been reported is hepatobiliary dysfunction. The etiological role of total parenteral nutrition in the production of cholelithiasis is not known, but various reports showed the association. The various effects of fasting, total parenteral nutrition and the underlying disease process on biliary lipid composition have not been studied in newborn infants.

Through a study of bile composition and concentration in the fasting infants, before and after total parenteral nutrition, the possible etiology of increased bile lithogenicity during parenteral nutrition can be elucidated. This in turn may help in the nutritional planning to decrease such a complication in the future.

In this study we have examined biliary lipids in neonates in an intensive care unit in an attempt to separate the effects of total parenteral nutrition from those of prolonged fasting.

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History of Parenteral Feeding

The concept of parenteral nutrition is not new and was known a long time ago. Trials on parenteral feedings were known as early as the seventeenth century. Hodder (1873) was the first to inject milk into the veins of a patient suffering from cholera. This was followed by trials of plasma protein infusions for feeding (1).

Van Slyke and Meyer (1913) reported their experience with intravenous infusion of casein hydrolysate into dogs. In the same year Henriques and Anderson reported their success with continuous intravenous infusion of an enzymatic hydrolysate of meat, glucose and salt into a goat for 16 days (1, 2). Alcohol was also one of the earliest nutrients infused intravenously. It was used in humans as early as 1891 by Atwater and Benedict. Clinical use was limited because of hepatotoxicity (1).

R. Elman (1940) reported his successful experience in infusing a solution containing glucose, electrolytes and amino acids (enzymatic hydrolysate of casein) into 35 adults and achieving positive nitrogen balance (3). Since then numerous studies have been made on the use of hydrolysate of casein in parenteral nutrition. A breakthrough in intravenous amino acid therapy was made by Japanese investigators who developed a method for the production of L-amino acids (1).

Research in nutrition was not limited to amino acids. Fat emulsions have undergone extensive research and trial. Courten (1679) was the first to inject warm oil of olive into the vein of a dog. However, the major advance was made by Yamakawa, Nomura and Sato

(1920) who prepared and used butterfat emulsions intravenously in animals and humans (1).

Many investigators like Stare and Geyer prepared and tested other fats. However, these trials were limited by the side effects encountered. Wrentlind and Schuberth in Sweden did extensive work on soybean oil. In 1961 they reported their success in obtaining emulsions of soybean stabilized with egg-yolk phosphatides (INTRA-LIPID) which proved to be safe and effective in humans (4, 5).

Progress was also made in using hypertonic glucose solutions to supply energy. The major advance was made by Dudrick (1968) who was the first to use hypertonic glucose (20%), together with electrolytes, amino acids, vitamins and trace elements through a central vein (6). Since that time many studies from all over the world have reported the successful use and understanding of parenteral nutrition.

Parenteral nutrition research also included infants and children, but to a lesser degree. One of the early successful reports on the use of parenteral feeding in infants was made by Heird, Driscol et al (1960). They administered an intravenous infusion of glucose, protein hydrolysate and ethanol through a peripheral vein in an infant with intractable diarrhea (7, 8). However, since that time little progress was made until 1968 when Dudrick and his colleagues introduced the use of hypertonic solutions given through central cannulas in the superior vena cava. Dudrick first treated an infant with gastrointestinal anomally (6). This was followed by rapid progress in the use of parenteral nutrition in infants and children.

Nutritional Requirements of Newborn Infants

The goal of feeding regimens for low-birth-weight infants is to obtain a prompt resumption of growth at a rate approximately equal to intrauterine growth rate. This rate of growth approximates that of the third trimester of intrauterine life (9) and is commonly equated with the rate of weight gain (10, 11). Poor nutrition in those infants is associated with an increased incidence of impaired growth and neuropsychological developments (10, 12).

I. Caloric Requirements of Newborn Infants

The basal metabolic rate of low-birth-weight infants is lower than that of full-term infants during the first week of life, but reaches and exceeds that of the full-term infant by the second week (9). Caloric requirements reach 50 - 100 Kcal/Kg/day during the first week of life and usually increase to 120 - 150 Kcal/Kg/day subsequently (9, 13). This energy requirement is partitioned between needs for resting expenditure, growth and storage (9, 13, 14).

II. Protein, Fat and Carbohydrate Requirements

There is considerable controversy over the requirements of proteins for premature infants, however, there is some agreement that it is about 3 g/Kg/day or 2.54 g/100 Kcal (9, 15). Although there is no known requirement for lipids in the infant diet, common practice dictates 40% to 50% of calories supplied by fat. A minimum of approximately 2.7% of total calories must be supplied by essential fatty acids (300 mg of linoleic acid per 100 Kcal) (9, 13, 16). Carbohydrates provide 40 - 50% of calories. It is important to know

that premature infants can adequately digest disaccharides, however, lactose digestion may not be efficient in the first few days of life (9, 16).

III. Electrolyte Requirements

The very low-birth-weight infant may require greater sodium intake during the period of active growth. The recommended requirements of Na+, K+ and Cl- is about 2 - 3 mMol/Kg/day each (17, 18). Calcium requirements for the low-birth-weight infant is higher than term infants and Fomon and co-workers have estimated that an intake of 158 mg/Kg/day are needed in 28 to 32 week infants (15).

IV. Trace Elements and Vitamins

The requirements for other electrolytes, trace elements and vitamins is shown in Table I. Because premature and low-birth-weight infants often consume fewer calories during early weeks of life, it is recommended that supplemental vitamins be given to those infants (9).

Methods of Nutrient Delivery

The advantages of early feeding in maintaining normal metabolism, growth and development are quite obvious. It has been shown that this approach may decrease the incidence of hypoglycemia, hyperbilirubinemia, dehydration, azotemia and electrolyte problems (19, 20, 21).

Oral feeding is preferred provided that there is a good sucking reflex and the infant is stable. The gestational age of such infants who can tolerate oral feeding is usually 32 weeks or older and they usually weigh 1300 to 1600 g (19). Care must be taken to avoid complications such as aspiration (22). If the baby is unable to suck,

but can tolerate enteric feeding, then tube feeding should be used. Babson has suggested the use of intermittent gavage feeding using soft rubber catheter (19). Pyati and co-workers have suggested the use of continuous drip nasogastric feeding (23). Because both procedures are thought to be associated with increased incidence of aspiration, Caillie and Powell have suggested the use of continuous nasoduodenal tube feeding (24). Others have also recommended the use of naso-jejunal tube feeding in order to supply greater calories. This avoids gastric distension and aspiration, and reduces disturbance to the patient (25, 26, 27). Unfortunately nasojejunal feeding is associated with some complications such as intestinal perforation (28, 29). Reimer and co-workers have suggested the use of a gastrostomy in children who will require tube feeding for long periods (30).

The above mentioned methods cannot be used in all infants, and in such cases parenteral feeding is to be used. This will be discussed in the following sections.

Indications for Parenteral Feeding in Neonates and Children

According to Dudrick and Ruberg the principle of parenteral nutrition is to provide adequate calories, nitrogen, water, electrolytes and vitamins (31). They summarized the indications for parenteral nutrition as "patients who cannot eat, should not eat or cannot eat enough" (31, 32).

In the neonates, the low-birth-weight infants are the usual candidates for parenteral feeding because of an increased incidence of disease processes. Gunn and co-workers and Shaw have pointed out the

benefits of parenteral nutrition in premature infants with respiratory distress syndrome (33, 34). Parenteral feeding is indicated in congenital cardiac anomalies associated with heart failure and also indicated in some intracranial injuries (32). One of the common conditions seen in premature infants requiring parenteral feeding is necrotizing enterocolitis. It has been demonstrated that parenteral feeding improves the outcome in patients with this condition (35). Another use of parenteral feeding in neonates is patients with surgical disorders of the gastrointestinal tract such as gastroschisis, omphalocele, short bowel syndrome and others (32, 36, 37, 38). Yu and co-workers and Shaw have suggested the use of parenteral nutrition in very low-birth-weight infants (34, 39). Parenteral feeding is also indicated in patients with hepatic failure, sepsis and malnutrition (30). Abel and co-workers (1973) and Baek and co-workers (1975) have demonstrated that parenteral nutrition is associated with improved survival and decreased morbidity in patients with acute renal failure (40, 41). Another indication for parenteral feeding is infants with intractable diarrhea. The mortality rate of these patients was high before parenteral feeding (42). Hyman and others have demonstrated that parenteral feeding improves survival in those patients (43). The use of parenteral feeding in the management of children with malignant disease has been pointed out by Filler and co-workers and others (44). Parenteral nutrition is also indicated in children with inflammatory bowel disease to improve growth and promote closure of fistulas (32, 36).

There are other conditions in which parenteral feeding is indicated such as trauma, burns, major surgery and others (32). In summary, parenteral feeding is a useful method of supplying energy to those patients who cannot tolerate enteral feeding, or need more energy than can be supplied by enteral feeding.

Complications of Parenteral Feeding

Parenteral feeding is widely used and regarded as an effective form of nutritional treatment in the presence of reasonable indications for its use. It is however associated with complications, like any other form of therapy. Many patients who require intravenous feeding are extremely ill and additional complications may be disastrous. It is however, only an awareness of the many potential dangers that makes this form of treatment relatively safe (45).

There are many potential complications associated with parenteral feeding and these complications are usually classified into four groups. In this discussion they will be classified into: (1)technical, (2) septic, (3) metabolic, and (4) hepatobiliary complications.

I. Technical Complications

Technical complications are the most common and many are potentially life endangering. Structures are small in the pediatric patient and there are many vital structures in the area of the thoracic inlet (30). Technical complications are usually seen with the use of catheters inserted into the superior vena cava. During catheter insertion, cardiac arrythmias, air embolism, pneumothorax, hemothorax or chylothorax may occur (32, 45, 46). Hydrothorax,

hydromediastinum and pericardial effusion have been reported as complications (47, 48). Paralysis of the diaphragm as a complication of central parenteral alimentation in children has been reported by Lam et al. and others (49). Thrombotic complications are commonly seen after prolonged use of central venous catheters. Mollitt and coworkers have reported a 7% incidence of superior vena caval thrombosis after prolonged parenteral feeding. They also pointed out that this complication is associated with increased morbidity and mortality (50). Other rare but possible thrombotic complications include intracardiac thrombi (51, 52) and coronary sinus thrombosis (53). These thrombotic complications may be reduced by the use of silicone rubber catheters instead of polyvinyl catheters (54). Other potential complications include catheter dislodgement and arterial puncture (45).

These complications are not commonly seen nowadays due to improvements in the technique of catheter insertion and care.

II. Septic Complications

Similar to technical complications, septic complications are frequent and may result in serious consequences. The incidence of catheter related sepsis varies from 1.3% to 5% depending on the series and criteria used to define sepsis (55, 56). Ryan and coworkers have suggested that the peak incidence of bacterial sepsis occurs at 2 weeks and fungal sepsis at 3 weeks (57). The incidence of catheter related sepsis depends on the care of the catheter and increases with improper handling of the catheter and connecting lines

(57, 58). The most frequent organisms causing sepsis are Staphylococcus aureus, S. epidermidis and Candida albicans. However, other organisms such as gram-negative bacteria and other fungi may be the cause of sepsis (59). Sanders and Sheldon have defined catheter sepsis as a clinical episode of systemic infection in which the primary source of infection is a parenteral nutrition catheter. They have also divided catheter sepsis into three categories: (1) proven, (2) clinical, and (3) questionable catheter sepsis (56). Proven catheter sepsis is established when the infection resolves with catheter removal and peripheral blood cultures are positive. Clinical catheter sepsis is made when symptoms resolve after catheter removal, even if blood cultures are negative. In questionable catheter sepsis, clinical symptoms do not resolve with catheter removal.

It has been shown that the incidence of septic complications markedly decreased following the introduction of TPN teams (56, 59). The major advantage of a team approach is the clear understanding of the cause of infection and experience in management of patients with catheter sepsis.

Fever, leukocytosis, or unexplained glucosuria, separately or in combination is often the first indication of sepsis related to central parenteral nutrition lines as pointed out by Coran and others (60). Infection is confirmed by blood cultures obtained through the central venous line and another venous site. At the same time the patient should be examined to exclude other sources of sepsis. Should indications of septicemia develop in a patient on parenteral

nutrition, certain measures should be taken, in addition to the usual cultures. The parenteral nutrition administration set and bottles should be removed and cultured (61). If there is no improvement in 8 hours, then the catheter should be removed and the tip of the catheter cultured (30). Some people prefer to change the catheter over a sterile guide wire to reduce the potential complications associated with catheter reinsertion (55). If the catheter is removed it is recommended to allow 24-48 hours to elapse prior to insertion of another central venous catheter (46). Systemic antibiotics are rarely indicated except in patients who are leukopenic.

III. Metabolic Complications

The pediatric age group is at special risk of developing metabolic complications because of the low reserves of fat, glucose and trace elements. Although some of the complications are unavoidable, most can be prevented by appropriate adjustments of the infusate and careful monitoring (54). Abnormalities of carbohydrate metabolism are seen mainly in small premature infants. Seashore reported his experience with six babies who developed hyperglycemia and persistent glucosuria after parenteral feeding. Five of the six babies were premature and weighed less than 2000 gm (62). His initial management consists of lowering the glucose concentration of the parenteral fluid and if no response is seen, exogenous insulin is used. Kaminski has pointed out that patients with a family history of diabetes mellitus are at a special risk of developing hyperosmolar, hyperglycemic, nonketotic dehydration (63). This complication may

result in certain morbidity and mortality and can be prevented by careful monitoring of blood and urine glucose. Hypoglycemia generally occurs when the rate of hypertonic glucose infusion is increased and then suddenly stopped (64). Premature infants and patients who are stressed are at greater risk of developing hypoglycemia and should be weaned over 8-12 hours. Fluid imbalance is seen more in premature infants. Hypovolemic dehydration may be the result of osmotic diuresis or from inadequate replacement of fluid losses. Fluid overload is commonly seen in infants receiving parenteral feeding. This is because more volume is given to provide adequate calories (62). Metabolic acidosis has been reported as a complication of amino acid therapy. However, it is rarely seen nowadays except in patients with renal or liver disease and premature infants (54). Seashore et al. and others have reported the occurrance of hyperammonemia as a complication of parenteral nutrition in infants and children (65). They have pointed out that low-birth-weight infants are at highest risk. They have also recommended the frequent measurement of blood ammonia during parenteral fluid therapy and that protein intake should be decreased if hyperammonemia occurs. Electrolyte imbalances secondary to inadequate or excessive infusions can occur and require frequent monitoring. Zinc, copper and chromium deficiencies have all been reported in patients on prolonged parenteral nutrition without supplementation (30). Essential fatty acids deficiency has been reported in patients receiving long-term fat-free parenteral nutrition, and it can be prevented by infusing fat emulsion

(INTRALIPID) (66). INTRALIPID is a relatively safe and effective therapy for supplying essential fatty acids and calories. However, its safety has been questioned in immature infants with compromised cardio-respiratory systems (67, 68). Levene et al. have speculated that in ill infants the emulsion becomes less stable and agglomeration of fat particles occurs which are filtered by the lungs (67). Vitamin deficiency is rare if adequate replacement is given, but vitamin toxicity due to excesses of Vitamins A and D has been reported (30). Calcium, magnesium and phosphate derangements have been reported in infants and children. The and his co-workers have reported a case of rickets in a preterm infant during parenteral feeding, and recovery was achieved by giving extra phosphorous supplementation (69). Klein et al. have reported three infants with Vitamin D-resistant rickets associated with parenteral nutrition (70). No abnormalities in serum calcium or phosphorous were found in these infants and all responded to doubling their Vitamin D intake. Metabolic complications of total parenteral nutrition in infants and children are numerous and frequent. Properly designed nutrient solutions, careful monitoring, knowledge of potential problems, and appropriate management are essential to avoid serious complications (62).

IV. Hepatobiliary Complications

The association between hepatobiliary dysfunction and total parenteral nutrition was first recognized in 1971. Peden et al. have described an infant who developed hepatosplenomegaly, cirrhosis and

cholestasis in association with total parenteral feeding (71). Beale and co-workers have shown that intrahepatic cholestasis occurred in 14 of 62 infants receiving parenteral nutrition, an incidence of 23%. They also pointed out that serial direct bilirubin levels are an indicator of cholestasis. In their study the mean time on parenteral nutrition to onset of cholestasis was 42 days. The very low-birth-weight infants (less than 1000 gm) appeared to be at increased risk of developing cholestasis with an incidence of 50% (72). Several reports appeared in the literature describing elevation of liver enzymes and bilirubin after parenteral nutrition (73, 74, 75). Vileisis et al. have advocated the use of direct bilirubin measurements as an indicator of hepatic status in infants receiving parenteral nutrition (75). However, it has been reported that the measurements of serum-sulfated lithocholate is a more sensitive indicator of cholestasis than either direct bilirubin or aminotransferases (76).

The etiology and pathogenesis of cholestasis occurring during parenteral nutrition is not known. Excessive amino acid input has been claimed to be a precipitating factor (77). Messing et al. have related cholestasis during parenteral therapy to excess nitrogen input, infection and bacterial overgrowth in the gastrointestinal tract (78). Other suggested etiological factors are imbalances of amino acids (79), sepsis (80) and hyperammonemia (81). Sondheimer and co-workers have studied cholestasis in premature infants on parenteral feeding and indicated that the small premature infant has a limited hepatic excretory function and this might be an important factor in

the susceptability of these infants to cholestasis associated with long-term parenteral nutrition (82). Roslyn and co-workers have proposed that the increased risk of gallbladder disease among patients on long-term parenteral feeding results from prolonged fasting. They have also indicated that fasting promotes hepatic secretion of cholesterol saturated bile as well as gallbladder stasis (83). Messing et al. have suggested that cholestasis and cholelithiasis occurring secondary to prolonged fasting and bowel rest during parenteral feeding, might be prevented by stimulating gallbladder contractions with intermittent oral administration of fat or protein (84).

Several reports have appeared in the literature describing the pathologic picture of the liver during prolonged parenteral nutrition. Benjamin has reported the pathologic findings in fifteen infants who were on long-term parenteral nutrition and developed hepatobiliary dysfunction (85). According to Benjamin, cholestasis is a universal finding and is both intracellular and intracanalicular. The portal areas become expanded with increased fibrous tissue. Pericholangitis is a common finding. Bile ductule proliferation is seen in severe cases. Pseudoacinar transformation, giant cell transformation and nodule formation may be seen. Cholelithiasis is one of the pathologic findings in some patients. Benjamin has suggested that the prolonged fasting results in disruption of the normal gastrointestinal mechanisms responsible for bile production and flow. A similar pathologic picture has also been described by Hodes and co-

workers (86), and Cohen and Olsen (87). The latter have indicated that early pathologic changes can be detected after five days of parenteral feeding. However, they have pointed out that most of the liver changes occurring within the first ninety days of total parenteral feeding are reversible.

Many reports have been published on the increased incidence of cholelithiasis associated with the prolonged use of total parenteral nutrition. The incidence of cholelithiasis in adult patients receiving long-term total parenteral nutrition is about 45% (88, 89). The increased risk of gallstones is not limited to adult patients, but also observed in premature infants (90), infants and children (91, 92) on long-term parenteral nutrition. Akierman et al. have described a preterm infant who developed gallstones after prolonged fasting and parenteral nutrition and when parenteral feeding was stopped and oral feeding started, the gallstones disappeared (93).

The etiology of the increased incidence of cholelithiasis during total parenteral nutrition is not known. Van der Linden and Nakayama have attributed the increased lithogenicity of bile to the infusion of fat emulsion (94). Gimmon et al. have studied the effect of parenteral and enteral feeding on bile composition in rats (95). They have found that continuous administration of the total parenteral nutrition solution increased bile lithogenicity in both parenterally and intragastrically fed rats. Using the same solution intermittently did not affect bile lithogenicity whether used parenterally or intragastrically. However, Gimmon's study has not been duplicated in humans.

Other suggested causes of increased incidence of gallstones during total parenteral therapy are gastrointestinal disease process (91), prolonged fasting (93) and sepsis (80). These studies and others have not been able to find out a definite cause for hepatobiliary dysfunction seen during total parenteral nutrition. Further studies are required to settle this problem.

Pathogenesis of Cholesterol Gallstones

Biliary tract diseases and especially gallstones are among the most common illnesses seen in North America, and they represent a major cause of morbidity and health care costs. About 10% of men and 20% of women in the United States have gallstones between the age of 55 to 64 (96). Gallstones are usually classified into cholesterol stones, which are the commonest, and pigment stones. The majority of cholesterol stones are mixed with bile acids, bile pigment, calcium and protein matrix. Cholesterol stones (both pure and mixed) constitute about 90% of gallstones (97).

Early attempts to understand the pathogenesis of cholesterol gallstones focused on events in the gallbladder. Gallbladder infection with exfoliation of inflammatory cells was thought to serve as a nidus for cholesterol precipitation (98). Then investigators began to pay more attention to the physicochemical properties of biliary lipids. The three major classes of lipids in human bile are cholesterol, bile acids and phospholipids. Cholesterol is virtually insoluble in water and is held in solution in bile by its association with the bile salts and phospholipids in the form of mixed micelles

(99, 100). Andrews and co-workers in 1932 have reported that cholesterol precipitation from bile is caused by a lowering of the bile salt content of the bile. They also pointed out that the infected gallbladder absorbs bile salts rapidly but cholesterol slowly. The normal gallbladder does not have such differential absorption (101). There are two major possibilities accounting for biliary cholesterol precipitation. Either the liver produces a bile supersaturated with cholesterol that precipitates in the gallbladder (or ducts); or the liver produces a bile of normal composition that is altered by the gallbladder (or ducts) to form an abnormal bile (102).

There are three stages in the formation of cholesterol gallstones. These include the production of abnormal bile, crystallization and growth (103).

I. Production of Abnormal Bile:

This is an important step in the formation of cholesterol gallstones. It involves alteration of biliary lipid proportions, resulting in an abnormal bile saturated with cholesterol (97). Admirand and Small have demonstrated that the solubility of cholesterol in bile depends primarily on the relative molar concentrations of bile acids, lecithin and cholesterol (104). They have presented the solubility of cholesterol in various aqueous mixtures of bile salt and lecithin on triangular co-ordinates. They also described a sharp line of separation between the chemical composition of bile from patients with and without gallstones. Cholesterol saturation in bile and the potential for precipitation,

occurs when cholesterol constitutes more than 10% of total lipids in bile (99, 104). Holzbach et al. have questioned the micellar zone limits, demonstrated by Admirand and Small, and suggested that it is smaller (105). They have also found that bile from patients with cholesterol cholelithiasis has a micellar zone similar to normals but differs compositionally in that there is a greater excess of cholesterol above saturation. Carey and Small have shown that the total lipid concentration is a predominant determinant of cholesterol saturation (106).

The lithogenic index has been proposed by Metzger et al. as a method for determining cholesterol solubility (107). It is the ratio of the actual amount of cholesterol present in a bile sample to the maximum amount of cholesterol that can be dissolved in the sample. A value greater than one indicates a bile supersaturated with cholesterol. Metzger has used the Admirand and Small triangular co-ordinates to calculate the lithogenic index. The lithogenic or solubility index can also be mathematically calculated according to the equation of Thomas and Hofmann (108). Another method for calculating the lithogenic index or percent cholesterol saturation is the use of the critical tables suggested by Carey (109). This method is simple and employs both relative and total lipid concentrations.

Although some studies have failed to separate normal from abnormal bile (110), the phase diagram suggested by Admirand and Small continues to serve a useful function. The use of the triangular co-ordinates emphasizes the fact that supersaturation of bile may result

from either an increase in the secretion of cholesterol or a decrease in the secretion of bile acids or phospholipid. However, little data is available to suggest the pathogenic importance of phospholipid secretion (98).

From the above data it seems that the critical step in bile saturation is either an increase in cholesterol or a decrease in bile acid secretion.

Increased Secretion of Cholesterol

Many factors can increase biliary cholesterol secretion and hence increase the risk of cholelithiasis. Genetic predisposition to cholesterol stone formation is seen in American Indian women. In these women biliary cholesterol secretion is increased (111). Cholesterol production is roughly proportional to the total body weight and obese individuals excrete an excess of cholesterol into bile (99, 112). A high caloric diet leads to increased secretion of biliary cholesterol (98). This might be due to an increased activity of hydroxymethylglutaryl CoA (HMG CoA) reductase, which is the rate-limiting enzyme in cholesterol synthesis. Excessive intake of cholesterol is not clearly associated with gallstone formation (98). The use of lipid lowering agents such as clofibrate is associated with increased biliary cholesterol secretion and gallstone formation (113). Increasing age and the use of estrogens have also been shown to be associated with increased biliary cholesterol secretion (99).

Decreased Secretion of Bile Salts

Bile salts are synthesized in the liver from cholesterol. Their synthesis involves nine steps and the first reaction is the rate-

limiting step, which is under the influence of 7 α-hydroxylase (114). The production of bile salts is under negative feedback control (115). Increased return of bile salts to the liver through the enterohepatic circulation will suppress bile salt synthesis. Bouchier has summarized the mechanisms for a reduced total body pool of bile acids (99). The first is an oversensitive feedback regulation. The second is a decreased efficiency of intestinal absorption which can result from interruption of the enterohepatic circulation. This can be seen in patients with ileal resection or bypass, regional ileitis, diarrhea (116), and T-tube fistula (98). The third mechanism is increased cycling frequency of bile salts within the enterohepatic circulation with normal feedback control. The forth mechanism is a decreased steady-state synthesis.

Although bile acid secretion can decrease in some situations, only in a few instances supersaturation and stone formation result from a relatively pure deficiency in bile acid secretion (98).

II. Crystallization of Cholesterol:

Saturation of bile with cholesterol does not necessarily lead to gallstone formation unless crystallization occurs. Crystallization can occur by either spontaneous or heterogeneous nucleation (117). Spontaneous nucleation requires marked supersaturation of bile to cause aggregation. The heterogeneous nucleation requires the presence of a nucleating agent (98). Agents potentially responsible for the seeding of the nidus upon which cholesterol precipitates include calcium salts, mucus, bile pigments, mucoproteins, bacteria, foreign bodies and cells detached from the gallbladder wall (97, 112).

III. Growth of Gallstones:

The development of gallstones requires a period during which microcrystals increase in size without being discharged from the biliary tree (112). Evans and Cussler have pointed out that the process of gallstone growth depends on aggregation (118). Many small cholesterol crystals agglomerate to form a single composite stone. This process is enhanced by small amounts of ionic calcium. Biliary stasis and infection may also contribute to the growth of stones (97). Conditions in which gallbladder contraction is impaired, such as diabetes mellitus, celiac disease, after vagotomy, and pregnancy, may account for the increased incidence of gallstone disease (98, 112).

In summary, gallstone formation seems to involve many factors. Cholesterol saturation seems to be a critical step in stone formation. However, other factors such as nucleation and stone growth are important. Gallbladder stasis and infection may also determine gallstone formation.

Effects of Fasting on Biliary Lipids

The association of cholestasis and cholelithiasis and total parenteral nutrition has been reported (91, 93). It was noted that the introduction of oral feeding and discontinuation of parenteral feeding resulted in the spontaneous disappearance of gallstones (93). These findings and others have led some investigators to suggest that prolonged fasting results in disruption of the normal gastrointestinal mechanisms responsible for bile production and flow (85, 91). Such

changes may lead to cholestasis and increased lithogenicity of bile. Rager and Finegold have suggested that early fasting and the lack of the normal gastrointestinal stimuli for bile formation and flow may be responsible for cholestasis seen in premature infants (119).

It has been suggested that during fasting, bile becomes more saturated with cholesterol, both in normal and gallstone patients (99). Williams and co-workers have studied the effect of fasting on bile lithogenicity in 19 normal men and 22 normal women (120). They have estimated the molar percentages of bile acids, phospholipid and cholesterol after random overnight fasts of 9, 12 and 16 hours. They have demonstrated that the duration of fasting determines the lithogenicity of bile which is more pronounced in women. A similar study was performed by Bloch et al. but the period of fasting was extended to 20 hours (121). This study has shown that maximum cholesterol saturation occurs after fasting for 10 to 20 hours but subsequently falls.

It seems that during fasting there is dissociation of cholesterol from phospholipid and the cholesterol to phospholipid ratio rises and bile becomes saturated (99). Mok and co-workers have suggested that the high saturation of bile during fasting may be caused by interruption of the enterohepatic circulation (122). This leads to severe reduction in bile acid secretion and marked cholesterol: phospholipid dissociation. However, they have pointed out that some of their patients were able to maintain a relatively high secretion of bile acids in the fasting state, which might be due to by-pass of the

gallbladder and maintenance of an intact enterohepatic circulation. It has been reported that during fasting there is an absence of neural and hormonal stimulation to the extrahepatic biliary tract and this leads to preferential flow of bile into the gallbladder (93). This was supported by several reports showing gallbladder distension during fasting and sepsis (123, 124). This leads to slowing of the enterohepatic circulation.

Although there is conflicting data on the effects of fasting on biliary physiology and bile composition, it is possible that prolonged fasting and the effect of the underlying disease may have a role in the increased lithogenicity of bile. This may be the result of slowing the enterohepatic circulation and cholestasis.

Biliary Lipid Metabolism in Infants and Children

Cholelithiasis is rare in infancy and slowly increases in incidence over the age of five. Girls and overweight children are at a higher risk (125, 126). Hemolytic anemias and congenital malformation of the biliary tree are now believed to be a less common cause of gallstones in children than was originally thought. Most gallstones of children are radio-opaque (126). Heubi and co-workers have studied biliary lipid composition in infants and children (127). They have found that normal infants and children had significantly lower biliary cholesterol content and cholesterol saturation than young adults. The low incidence of gallstones in children has been attributed to a decreased percentage saturation of cholesterol as compared to adults (127, 128). Bile salt metabolism was studied in

premature (129) and term (130) infants. Both studies have established that infants are capable of bile salt synthesis. However bile salt pool size is smaller than that of the adult. This reflects the functional maturity of the liver. It has also been shown that medications can influence bile salt production (129).

In recent years several reports have shown that the incidence of cholestasis in infants and children is increasing (72, 78, 82). Nakai and co-workers have indicated that bile stasis was associated with prematurity, esophageal atresia, duodenal atresia, lower small bowel obstruction and sepsis (131). They have suggested that biochemical dysfunction of liver cells, duct obstruction and disturbance of hepatic blood flow may be the causes of bile stasis. Biliary lipid composition was studied in infants (132) and children (133) with cholestasis. Biliary lipid concentrations were extremely reduced and bile acid concentrations were below the critical micellar concentration. Cholesterol saturation was found to be higher than controls. These changes may account for the higher incidence of gallstone formation associated with cholestasis. Becker and co-workers have pointed out that changes in biliary lipid composition seen in cholestasis may be the result of disturbance in the synthetic function of liver cell or interruption of the enterohepatic circulation (133). Biliary lipid composition was also studied in children with cystic fibrosis (134, 135). Children with cystic fibrosis have an increased incidence of gallstones. Biliary lipids of untreated children show an excess cholesterol and decreased bile

salts. It is suggested that chronic fecal bile acid loss may produce a contraction of bile acid pool and lead eventually to a reduction of intraduodenal bile acid concentrations. These changes respond to pancreatic enzyme therapy.

Biliary lipid composition is also disturbed in infants and children with ileal resection, short bowel syndrome, intractable diarrhea and other conditions (128).

OBJECTIVES

The objectives of this study are as follows:

1. To determine the effect of prolonged fasting on biliary lipids in neonates.

2. To determine the effect of total parenteral nutrition on biliary lipids in neonates.

3. To determine the effect of liver immaturity on biliary lipids.

MATERIALS AND METHODS

Clinical Material

This study was done on infants in the neonatal intensive care units of the University of Alberta Hospitals and the Royal Alexandra Hospital. Patients were selected on the basis of method of nutrition, birth weight and underlying disease process. Neonates on oral feeding were excluded. Neonates weighing less than 700 g at birth were also excluded for technical reasons. Neonates who were on parenteral feeding for less than two weeks were not included. Infants who were fasting for 3 days or more, or on total parenteral nutrition for 2 weeks or more were included in the study.

The study was approved by an ethical review committee at the University of Alberta and Royal Alexandra Hospitals. Parental consent was obtained after a full explanation of the details of the study as was the consent of a neonatologist familiar with but not involved in the study.

Following the previous criteria, thirty neonates were included in this study, with a mean gestational age of 33.6 weeks (range 26 - 41 weeks) and a mean birth weight of 1844 grams (range 700 - 3360 grams).

On the basis of the method of nutrition and the duration of nutrition, patients fell into three groups (Table II).

Group I

This group consisted of thirteen neonates (8 males and 5 females) who were fasting (no oral feeding) for three to six days and

maintained on dextrose-electrolyte solution intravenously. The mean gestational age is 33.3 weeks (range 27 - 39 weeks) and the mean birth weight is 1891 grams (range 960 - 3180 grams).

Group II

This group consisted of eight neonates (4 males and 4 females) who were on total parenteral nutrition for up to two weeks and no oral intake. The mean gestational age is 33.7 weeks (range 26 - 40 weeks) and the mean birth weight is 1784 grams (range 930 - 2650 grams).

Group III

This group consisted of nine neonates (4 males and 5 females) who were on total parenteral nutrition for a period of three to eight weeks with no oral intake. The mean gestational age is 33.8 weeks (range 26 - 41 weeks) and the mean birth weight is 1858 grams (range of 700 - 3360 grams).

The clinical data for each group are shown in Tables III, IV, V and VII. These tables indicate the three groups are comparable in terms of gestational age, birth weight, sex distribution and underlying disease process including the incidence of neonatal jaundice. These tables also show that the most common disease processes requiring withdrawal of oral intake and initiation of parenteral nutrition in these infants are prematurity, necrotizing enterocolitis, sepsis and respiratory distress syndrome.

Total Parenteral Nutrition Solutions

The total parenteral nutrition solutions used in these infants consisted of an amino acid solution (Travasol), fat emulsion (INTRA-

LIPID) and glucose. It also contained electrolytes, vitamins and trace elements. Parenteral feeding was increased gradually and stabilized to supply 2 - 2.5 g/Kg/day of proteins, 2 - 3 g/Kg/day of fat and 12 - 15 g/Kg/day of glucose.

Gallbladder Ultrasonography

An ultrasound examination of the gallbladder was performed on infants who were on total parenteral nutrition for four weeks or more. Six neonates from Group III had an ultrasound of the gallbladder.

Duodenal Intubation and Collection of Bile Specimens

Duodenal bile from each patient for lipid analysis was obtained by duodenal intubation using a 5 - 8 French soft rubber catheter (Argyle tube). First the patient was positioned on his or her right side and then the appropriate size tube was introduced either nasally or orally into the stomach. By careful manipulation, the tube was advanced into the duodenum. The position was confirmed by one of three processes: (1) a Ph of 6.5 or more and yellow color of the aspirate (bile) (129, 135), (2) a plain x-ray of the abdomen or, (3) by intraoperative positioning. After confirmation of the tube position, the gallbladder was stimulated by an amino acid solution (10% Travasol) introduced through the tube into the duodenum (136, 137, 138). A dose of 0.5 - 1.0 ml/Kg was followed for all infants. The tube was then clamped for about 20 minutes after which time bile was aspirated from the duodenum manually using a 10 ml syringe over 1 - 2 hours. Bile was collected into a plain tube and taken immediately

to the laboratory. Specimens were then mixed and extracted and then refrigerated until analysis time. The volume of bile samples was not large and ranged around 1 - 3 ml.

Biliary Lipid Determinations

Biliary cholesterol, phospholipid, total bile acids and total lipids were determined for each bile specimen.

Phospholipids were determined by the method of Sunderman and Sunderman (139). The principle of the technique depends on the extraction of the phospholipid phosphatidylcholine (Lecithin) with a mixture of chloroform and methanol. An aliquot of the extract (chloroform layer) is evaporated to dryness and Lecithin is digested with sulfuric acid and hydrogen peroxide. Phosphorous in the digested extract reacts with acid molybdate solution to form phosphomolybdic acid. The phosphomolybdic acid is reduced by aminonaphtholsulfonic acid to form a blue color with an intensity proportional to the concentration of phosphorus.

The procedure of extraction was done as follows:

(1) 1.0 ml of bile was added drop by drop to 22.0 ml of chloroform-methanol mixture while mixing on the vortex for five minutes.

(2) The tube was stoppered and shaken for 30 seconds and brought to 25.0 ml by adding 2.0 ml of chloroform-methanol mixture. Then it was allowed to stand for five minutes.

Then phospholipids were determined as follows:

(1) 5.0 ml of dilute sulfuric acid was added to the mixture and the tube was inverted 10 times and then allowed to stand for 10 minutes.

(2) The mixture was then centrifuged at 2000 rpm for 15 minutes and this separated the lower chloroform phase containing the lipids.

(3) 5 ml of the extract was transferred to a digestion tube and evaporated to dryness.

(4) 2.5 ml of 5N sulphuric acid was added to the mixture.

(5) A blank was set up using only 2.5 ml of the 5N sulphuric acid. A standard sample was prepared by transferring 0.5 ml of the phosphate standard solution to a digestion tube and adding 2.5 ml of the 5N sulphuric acid.

(6) Then the tubes were slowly boiled.

(7) After a brown or black color appeared and white fumes were rising in the tubes, the tubes were cooled slightly and five drops of 30% hydrogen peroxide were added. Heating was continued until the contents became colorless and if not hydrogen peroxide was added and heating continued. Heating was continued for 15 minutes after the contents became colorless.

(8) Contents of each tube were diluted with a few ml of deionized water and cooled to room temperature.

(9) The contents were transferred to a 25 ml volumetric flask by filtering through glass wool, with repeated washings so that the flask was about half full.

(10) 2.5 ml of 2.5% ammonium molybdate solution and 1.0 ml of aminonaphtholsulfonic acid reagent were added and contents diluted to the 25.0 ml mark with deionized water, mixed and allowed to stand for five minutes.

(11) The absorbance was read on the Unicam Spectrophotometer at 675 nm.

(12) Calculations:

$$\text{Phospholipid mg/100 ml} = \frac{\text{optical density (unknown)}}{\text{optical density (standard)}} X_{14} \times 25$$

1mM phospholipid - 793 mg/L

Enzymatic determination of total bile acids in bile was carried out using the method of Engert and Turner (140). The principle is based on the conversion of the 3-β-hydroxy bile acids to the 3-Keto bile acid by the enzyme 3-α-hydroxysteroid dehydrogenase, with concomitant reduction of β-nicotinamide adenine.

Reagents were:

1. Buffer, 0.1M sodium pyrophosphate
2. Hydrazine sulphate 1.0M (3.4 ml 95% hydrazine plus 1.5 ml concentrated sulphuric acid, and the volume made up to 100 ml with deionized water).
3. β-Nicotinamide adenine dinucliotide solution (NAD) 6.8 mM.
4. Enzyme preparation: 5 mg hydroxysteroid dehydrogenase plus 1.0 ml buffer solution.
5. All specimens and standards were run in duplicate and assayed as outlined in Table VI. The samples were incubated at 37°C for 60 minutes and then cooled to room temperature. Specimens were read on Beckman DU-8 spectrophotometer at 340 nm. A standard graph was constructed by plotting mM concentration of chenodeoxycholic acid standards agains optical density.

Total bile acids μ mol/ml = concentration in tube X $\frac{1000}{\text{volume of specimen}}$

Cholesterol was determined by the enzymatic colorimetric method (using the Abbott Bichromatic Analyser - ABA - 100) (141, 142).

Principle

Cholesterol esters are hydrolysed to free cholesterol by cholesterol esterase. The free cholesterol produced is oxidized by cholesterol oxidase to cholest-4-en-3-one with the production of hydrogen peroxide. Hydrogen peroxide couples oxidatively with 4-aminophenazone and phenol in the presence of peroxidase to yield a red quinoneamine dye. The intensity of the color formed is proportional to the cholesterol concentration and can be measured spectrophotometrically at 500 nm.

Reagents:

Reagent composition	Final Concentration
1. Tris buffer pH 7.7	100 m mol/L
2. Magnesium asparate	50 m mol/L
3. 4-aminophenazone	1 m mol/L
4. Phenol	6 m mol/L
5. 3,4-dichlorophenol	4 m mol/L
6. Hydroxyprolyethoxy-n-alkanes	0.3%
7. Cholesterol esterase	> 0.4 IU/ml
8. Cholesterol oxidase	> 0.25 IU/ml
9. Peroxidase	> 0.2 IU/ml

Instrument parameter:

1.	Filter	500/600
2.	Incubator	30°C
3.	Mode	End point
4.	Reaction direction	up
5.	Analysis time	10 minutes
6.	Carousel revolution	2
7.	Syringe plate	1:101
8.	Sample size	5µL
9.	Decimal setting	00.00
10.	Zero	0000

Data Analysis

The data collected was analysed in the following ways:

1. Molar percentage of each biliary lipid:

This is done by adding the concentration in mmol/L of cholesterol, phospholipid and total bile acids and finding the percentage of each one of them in relation to the total mmol/L. For example:

$$\text{Cholesterol molar percentage} = \frac{\text{cholesterol (mmol/L)}}{\text{Total mmol/L}} \times 100$$

2. Total biliary lipid concentration in g/dL was estimated by converting the concentration in mmol/L to g/dL using the molecular weights of 491 for mixed bile salts, 775 for biliary lecithin and 387 for cholesterol. Then the concentrations of the three lipids are

added. For example the cholesterol concentration in g/dL is estimated as follows:

Cholesterol (Chol) = Chol in mmol/L X 387 = mg/L

$$\text{Chol in g/dL} = \frac{\text{Chol in mg/L}}{1000 \text{ X } 10} = \text{g/dL}$$

3. The molar percentages of cholesterol, phospholipid and total bile acids were plotted on the triangular coordinates of Admirand and Small (104). The saturation index was determined using the Admirand and Small triangular coordinate as pointed out by Metzger (107). This is illustrated in Figure IX.

4. Statistical Analysis:

The following statistical methods were used throughout this study.

a) Student's T-test was used in the comparison of unpaired data. This was used in the analysis of the molar percentages and total concentration of biliary lipids between the groups.

b) The chi-square (x^2) test (using the 2 X 2 contingency table) was used to analyse the incidence of jaundice in those infants who had and those who did not have parenteral nutrition.

c) The linear regression equation was used to analyse the association of cholesterol molar percentage and the duration of fasting (Fig. I).

RESULTS

Table VII shows the incidence of neonatal jaundice in group I, II, and III. In group I, 10 neonates out of 13 developed jaundice. In group II, 5 neonates out of 8 developed jaundice and 3 out of 9 neonates develped jaundice in the third group. There is no significant difference in the incidence of jaundice between the infants not receiving TPN (Group I) and those receiving TPN (Groups II and III). It appeared that jaundice was physiologically due to liver immaturity, as it appeared early in life and resolved with time. It did not appear to be related to parenteral nutrition.

The biochemical data for group I, II and III are shown on tables VIII, IX and X. Each table shows the levels of cholesterol, phospholipid and bile acids in mmol/L and the percentage of each in relation to the total lipids. In group I, two neonates fasted for three days, four for four days, six for five days and one neonate fasted for six days. Neonates in group II were on parenteral nutrition for two weeks with no oral intake. In group III all neonates were not on any oral intake. One neonate had parenteral nutrition for three weeks, four for four weeks, three for six weeks and one for seven weeks. Table XI compares the total biliary lipid concentration in g/dL between all groups. The mean of total biliary lipid concentration is 0.117 g/dL for group I, 0.248 g/dl for group II and 0.077 g/dL for group III. It is clear that the lowest total biliary lipid concentration is seen in group III.

Figure I is a graph which shows the relation between the percentage of cholesterol in bile (as a percentage of total mmol/L) to

the duration of fasting in days. Thirteen neonates in group I who were fasting for 3 - 6 days, were plotted in this graph. It is clear that the relation between the percent cholesterol in bile (of total mmol/L) and the duration of fasting is linear. The correlation coefficient (r) for this graph is 0.90. This graph shows that after three days of fast, cholesterol saturation in bile increases to supersaturated levels.

Figure II is a bar graph which compares the mean percent cholesterol (of total mmol/L) of all groups. This graph shows that bile is saturated with cholesterol in groups I and III and borderline in group II. There is no statistical difference between group I and III. However there is a significant difference between group II and III ($p < 0.01$). Figure III is a bar graph which compares the mean percent total bile acids (percentage of total mmol/L) of all three groups. It appears that the percentage of bile acids in relation to total biliary lipids is low in groups I and III. It is high in group II. The difference between groups I and II, and II and III is statistically significant. Figure IV is a bar graph which compares the mean percent phospholipid (percentage of total mmol/L) of all three groups. Again groups I and III seem to be similar in that they both have a higher percentage of phospholipid in bile compared to group II. There is no significant difference between groups I and III. However both show a significant difference in relation to group II.

From figures II, III and IV it appears that groups I and III are similar. Group II appears different. Also group I and III show an

increased percentage of cholesterol in their bile and decreased percentage of bile acids. In other terms it appears that cholesterol solubility is significantly decreased in the fasting neonates and in the neonates who were on parenteral nutrition for 3 - 8 weeks. Neonates who were on parenteral feeding for only two weeks did not show significant adverse changes.

Figure V compares the total biliary lipid concentration in g/dL between the three groups and relates them to the adult range. It is obvious that the total biliary lipid concentration is low in the newborn infants and lies below the adult range. However a few infants in the second group produced enough biliary lipids to reach the adult range. Figure VI is a bar graph of the means of total biliary lipid concentration (g/dL) of the three groups. This graph shows clearly that total biliary lipid concentration is low in groups I and III and near the normal adult range in group II. Again groups I and III are similar and there is no significant difference between their total biliary lipid concentration. However each one of them differs significantly from group II.

The fall of the total biliary lipid concentration in the fasting neonates and the neonates on prolonged parenteral feeding (3 - 8 weeks) is significant as compared to that of neonates on parenteral feeding for two weeks.

When total biliary lipid concentration is broken down into the three components namely, cholesterol, phospholipid and total bile acids, it is apparent that cholesterol and phospholipid concentrations in bile change very little in the three groups. Total bile acid

concentrations shows a large increase in Group II when compared to Groups I and III and accounts for the increase in total biliary lipid concentrations seen in Group II. This is shown in figure VII.

The critical micellar concentration (CMC) or minimum concentration at which micelles start to form for bile acids in bile is 0.9 - 2.2 mM with a mean of 1.45 mM (114). The neonates in Group I showed bile acid concentrations below the CMC in 5 of 13 specimens. In Group II where bile acid concentration was significantly higher, all bile specimens were at or above the CMC. In Group III only two specimens were at the lower range of the CMC for bile acids and the remaining 7 specimens were clearly below the CMC as shown in figure VIII.

The Admirand and Small triangular coordinate (104) is shown in figure IX. This figure shows that seven out of thirty neonates fell out of the micellar zone and the lithogenic index could not be estimated by the method of Metzger (107) for them.

Six infants from group III, who were on total parenteral nutrition for more than 4 weeks, had an ultrasound of the gallbladder done on them. This showed sludge formation in the gallbladders of three infants.

DISCUSSION

In this study, patients were grouped into three groups on the basis of the method of nutrition used and duration of parenteral nutrition. All the infants were fasting in terms of oral intake. The three groups are comparable with regards to gestational age, sex distribution, birth weight and underlying disease process. The indications for the use of parenteral nutrition in groups II and III are prematurity, necrotizing enterocolitis, respiratory distress syndrome, sepsis, congenital anomalies of the gastrointestinal tract, congenital heart disease and neurological problems. These indications are consistent with the reported indications (34, 39 35, 30, 36, 38, 32). The parenteral nutrition solution used was maintained to supply enough calories, proteins, carbohydrates, fats, electrolytes, vitamins and trace elements in accordance with the reported requirements (9, 13).

Bile was obtained by duodenal intubation. This bile is a representative sample of hepatic bile. Vlahcevic and co-workers have shown that duodenal bile is also similar and representative of gallbladder bile (143). The stimulation of gallbladder contraction has been shown to occur with intraduodenal infusion of amino acid solution by Ertan et al., Duane et al. and Murthy et al. (138, 137, 136). Bile analysis was done using the standard methods outlined and any doubtful results were repeated. The clinical and laboratory data showed that the incidence of jaundice was the highest in group I

(fasting infants maintained on dextrose-electrolyte solution) and the lowest in group III (infants on parenteral feeding for 3 - 8 weeks). However there is no statistically significant difference in the incidence of jaundice between those infants who were not on parenteral nutrition and those on parenteral nutrition, i.e. group I and group II plus III. From the clinical and laboratory picture it appeared that this type of jaundice is physiological. It disappeared with time and it did not appear to be related to parenteral feeding. Gartner and Lee have pointed out that physiologic jaundice in the premature infant is more severe than the full-term infant, and more prolonged (144). Normal bilirubin concentrations in premature infants may not be reached in many cases until the end of the first month of life. In some of the infants in this study physiologic jaundice continued for 20 - 30 days of life.

Biliary lipid concentration in all infants studied was consistently low as compared to the adult values. However our results are consistent with those reported by Tazawa et al. (132). Also we had compared biliary lipid composition of the infants in this study with bile composition of an infant obtained by needle aspiration from his gallbladder (not included in the results) and found that the values are consistent. Total biliary lipid concentration (g/dL) is also consistently low and all infants fell below the physiologic range of adults except three infants in group II (two weeks of parenteral feeding). This adult physiologic range extends between 0.3g/dL to 30.0 g/dL as demonstrated by Carey (109). Becker and co-workers have

reported that the total biliary lipid concentration in g/dL is low in the bile of children with cholestasis (133). Their results were consistent with our results.

The incidence of gallstones is low in infants and children as pointed out by Strauss and Moosa (125, 126). This has been attributed to a decreased molar percentage of cholesterol in the bile of infants and children as compared to adults (127, 128, 145). However the incidence of gallstones is high in infants and children who are sick and on total parenteral nutrition. Whitington and Black (90), Benjamin (91) and Callahan et al. (146) have reported the increased incidence of cholelithiasis in infants on parenteral nutrition. In this study six infants from group III, who had parenteral feeding for more than four weeks, had an ultrasound of the gallbladder done on them. Three of the six infants showed sludge formation in the gallbladder. These three infants had total parenteral nutrition for 7 weeks, 6 weeks and 4 weeks consecutively. The molar percentage of cholesterol in these infants is high. The reasons behind the increased incidence of gallstones associated with total parenteral nutrition are not known. Van der Linden and Nakayama have attributed that to the infusion of fat emulsion (94). Zarif et al. have suggested that L-amino acids and dextrose solutions might be the inducing factor (147). However other reports have shown that hepatobiliary dysfunction can occur regardless of the type of solution used (148). Others have suggested gastrointestinal dysfunction (91), prolonged fasting (93) and sepsis (80). Math has attributed the

increased lithogenicity of bile and cholestasis to the high osmolarity of parenteral fluid used (149). He has pointed out that this will lead to decreased water content of bile and hence cholestasis. He has suggested that supplementary oral feedings with plain water to infants and children receiving total parenteral nutrition may probably help in preventing the development of hepatobiliary complications. Infants in group I who were fasting and maintained on dextrose-electrolyte solution showed a linear increase of their biliary cholesterol molar percentage with the duration of fasting in days. After a three day fast their bile became saturated with cholesterol. When cholesterol molar percentage is compared in all groups it appeared that the molar percentage of cholesterol is high and above saturation level in those infants who were fasting with no parenteral feeding (group I) and those infants who were fasting and maintained on total parenteral nutrition for 3 - 8 weeks (group III) although there was no significant difference between these two groups of infants. Infants who were fasting but received parenteral nutrition for two weeks (group II) showed a significant lowering of cholesterol molar percentage as compared to group III.

The molar percentage of total bile acids showed a significant decrease in those infants who were fasting (group I) and those who were fasting and maintained on total parenteral nutrition for 3 - 8 weeks (group III) as compared to fasting infants who had total parenteral nutrition for only two weeks (group II).

The above data suggests that fasting seems to influence the composition of biliary lipids. In the fasting infants, cholesterol

solubility decreased as the fasting period increased. Fasting might have changed the relative molar percentages of biliary lipids so that cholesterol molar percentage was increased and total bile acids molar percentage was decreased. A similar effect was seen in the fasting infants maintained on prolonged total parenteral nutrition for three to eight weeks. However, it appeared that in the fasting infants, the introduction of total parenteral nutrition might have exerted a temporary beneficial effect on biliary lipid composition as seen in group II infants. This effect of decreased cholesterol molar percentage and increased total bile acid molar percentage seemed to disappear as the total parenteral nutrition was continued for three to eight weeks with continued fasting. This change in biliary composition occurring after prolonged parenteral nutrition might be attributed to the effect of prolonged fasting. Bile composition appeared to be comparable in the fasting infants and in infants maintained on prolonged total parenteral nutrition. The beneficial effects of total parenteral nutrition during the first two weeks of therapy are difficult to explain. Total parenteral nutrition may indirectly stimulate the liver cells to produce more bile acids by supplying more energy to the liver cells. However, this is only a hypothetical explanation which needs more studies to support it.

The effect of fasting on biliary lipid composition has been studied in the adult population. Williams and co-workers have demonstrated that the cholesterol molar percentage increases during fasting and bile becomes more lithogenic (120). Bouchier has

suggested that cholesterol dissociates from phospholipids during fasting and bile becomes saturated with cholesterol (99). The increased cholesterol molar percentage and the decreased total bile acids molar percentage occurring during fasting might be due to the interruption of the enterohepatic circulation. This has been suggested by Mok et al. (122). This interruption of the enterohepatic circulation leads to severe reduction in bile acid secretion and marked cholesterol to phospholipid dissociation. Akierman and his co-workers have suggested that during fasting there is a preferential flow of bile into the gallbladder due to the absence of neural and hormonal stimulation of the extrahepatic biliary tree (93). They have also pointed out that the introduction of oral feeding leads to increased cholesterol solubility. Messing and his co-workers have suggested that cholestasis and cholelithiasis occurring secondary to prolonged fasting and bowel rest during parenteral feeding, might be prevented by gallbladder stimulation with intermittent oral administration of fat or protein (84).

One infant who was not included in the results was followed during the course of total parenteral nutrition. His duodenal bile was obtained after four weeks of total parenteral nutrition. The biochemical analysis of his bile showed a high cholesterol molar percentage which was above 13% and a low total bile acid molar percentage (55%). Subsequently this infant was started on oral feeding (nasoduodenal tube feeding) together with parenteral nutrition. After two weeks of combined nutrition, duodenal bile was

obtained which showed on analysis a decrease in cholesterol molar percentage (to 7.0%) and an increase in total bile acid molar percentage (to 68.3%). The introduction of oral feeding had also produced an increase in the total biliary lipid concentration of this infant. Before the introduction of oral feeding, the total biliary lipid concentration was 0.098 g/dL and this increased to 0.333 g/dL after two weeks of oral feeding. This suggests that prolonged fasting disturbs the normal biliary physiology leading to an increased lithogenicity of bile. It seems that the introduction of oral feeding results in the normalization of biliary physiology and improvement of biliary lipid composition.

Biliary lipid concentration (expressed in g/dL) was significantly low in the fasting infants (group I) and in infants who were fasting and maintained on prolonged parenteral nutrition for three to eight weeks (group III) as compared to those infants who were fasting and had parenteral nutrition for only two weeks (group II). However, there was not a significant difference between group I and group III. Again it seems that in both groups fasting may be the factor behind the decreased concentration of biliary lipids.

Carey and Small have shown that the total biliary lipid concentration is a predominant determinant of cholesterol saturation (106). Tazawa et al. and Becker et al. have reported that biliary lipid concentration is extremely reduced in infants and children with cholestasis (132, 133). These changes may be the result of disturbance in the synthetic function of the liver cell or interruption of the enterohepatic circulation.

In this study the reduction of total biliary lipid concentration (g/dL) seen in the fasting infants and in infants who were on prolonged total parenteral nutrition (3 - 8 weeks) was also associated with changes in the composition of biliary lipids. Biliary lipids showed an increased cholesterol molar percentage and a decreased total bile acid percentage when the total biliary lipid concentration dropped as seen in groups I and III. The decrease in lipid concentration and the change in molar percentage composition in these infants might be the result of changes in biliary physiology induced by fasting.

A consequence of the very dilute bile and low bile acid content seen in the infants in Groups I and III is that their bile acid level is below the critical micellar concentration for bile acids. The critical micellar concentration of bile acids in bile is the level below which there are insufficient bile acid molecules present to form micelles to solubilize cholesterol. Thus many infants in Groups I and III did not have enough bile acid molecules in their bile to solubilize cholesterol.

When the molar percentages of cholesterol, phospholipids and total bile acids of the infants in all groups were plotted on the triangular coordinate of Admirand and Small (104), the bile of seven infants showed an abnormal pattern. The plot of these seven infants showed a deviation from the micellar zone and lithogenic (saturation) index could not be estimated according to the method of Metzger (107) as illustrated in Fig. IX. The saturation index of the bile of these

infants could not also be determined by the Thomas and Hofmann equation (108). The bile of these seven infants showed a very significant drop in the molar percentage of total bile acids as compared to the adult population. It seems that there are several factors which contributed to these changes in biliary lipid composition. The first possibility is liver immaturity in these premature infants which is associated with decreased bile acid production. The second possibility is the effect of fasting which may be associated with interruption of enterohepatic circulation and hence decreased hepatic synthesis of bile acids. This has been pointed out by Mok et al. (122). Roy et al (134) and Goodchild et al. (135) have pointed out that the interruption of the enterohepatic circulation seen in infants and children with cystic fibrosis leads to a decreased bile acid production. The third possibility is that infants may have a different micellar zone than that of the adults.

In summary, it seems that newborn infants react to the effect of fasting and disturbed gastrointestinal physiology in varying ways. In this study it appears that this reaction is expressed in changes in biliary physiology leading to changes in biliary lipid concentration and composition. Although the effects of total parenteral nutrition are not clear, it appears that there is an early and temporary beneficial effect.

CONCLUSIONS

There are several conclusions that can be drawn from this study:

1. The neonatal liver is unable to produce a bile capable of solubilizing cholesterol beyond a three day fast (no oral intake). Bile becomes increasingly saturated with cholesterol after the three day fast. This is seen in infants who were not on parenteral nutrition.

2. The introduction of total parenteral nutrition appears to exert a lowering effect on the molar percentage of biliary cholesterol and a raising effect on the molar percentage of biliary total bile acids. This effect appears to last for two weeks. The reason behind this apparent beneficial effect of total parenteral nutrition is not known. After two weeks of parenteral feeding it appears that the effects of prolonged fasting abolish the beneficial effect of total parenteral nutrition. This results in increased lithogenicity of bile.

3. Total biliary lipid concentration expressed in g/dL appears to be affected by fasting. Fasting seems to decrease the capacity of the liver to produce biliary lipids leading to a decreased concentration of them in bile. The beneficial effect of total parenteral nutrition in the first two weeks of therapy is shown in the increased concentration of all biliary lipids. The above mentioned changes do not appear to be influenced by liver immaturity and it appears that fasting is a more influential factor.

4. The incidence of sludge formation in the gallbladder (presumably leading to cholelithiasis)increases with prolonged fasting associated

with total parenteral nutrition. The increased incidence seems to be more apparent after four weeks of fasting during parenteral feeding.

5. Neonates on long term total parenteral nutrition with no oral intake secrete a very dilute bile with an insufficient concentration of bile acid molecules to form micelles wherein cholesterol is solubilized. This finding may explain some of the adverse hepato-biliary changes associated with long-term parenteral nutrition.

Table I

NUTRITIONAL REQUIREMENTS FOR PREMATURE INFANTS*

NUTRIENT	PREMATURE INFANT, REQUIREMENTS PER 100 K cal
Protein	2.8 g
Fat	5.1 g
Carbohydrate	11.5 g
Vitamin A	260 IU
Vitamin D	63 IU
Vitamin E	1.9 IU
Vitamin K	9 µg
Vitamin C	8 mg
Thiamin	78 µg
Riboflavin	94 µg
Niacin	1250 µg
Vitamin B6	63 µg
Folic Acid	16 µg
Pantothenic Acid	470 µg
Vitamin B12	0.3 µg
Biotin	2.5 µg
Inositol	6 mg
Calcium	156 mg
Phosphorous	78 mg
Magnesium	10 mg
Iron	0.2 mg
Iodine	8 µg
Copper	100 µg
Zinc	0.65 mg
Manganese	160 µg
Sodium	1.7 mEq
Potassium	2.8 mEq
Chloride	2.4 mEq

*Modified after American Academy of Pediatrics

Table II

CLINICAL MATERIAL

	GROUP I	GROUP II	GROUP III
Type	Control	TPN for 2 wk	TPN 3 - 8 wk
no. of neonates	13	8	9
Nutrition	Dextrose-Electrolyte	TPN	TPN
Gestational age (wk) Mean ± SEM	33.3±1.2	33.7±1.6	33.8±1.8
Birth Wt. (g) Mean ± SEM	1891±201	1784±213	1858±297
Wt. (g) Mean ± SEM	1938±192	2070±222	2651±356

CLINICAL DATA - GROUP I (Control)

Table III

PATIENTS	DIAGNOSIS	Gestational Age (wks)	Sex	Birth Weight (g)	Weight at Sampling (g)	Age at Sampling (days)	Apgar Score	
							1 min.	5 min.
No. 1	Prematurity and NEC Sepsis	35	F	1950	1900	4	4	8
No. 2	IUGR and Birth Asphyxia	36	M	1800	1750	4	0	3
No. 3	Prematurity and Twin Sepsis	32	M	1480	1500	3	4	8
No. 4	RDS & IUGR NEC	37	M	2600	2550	4	4	6
No. 5	Prematurity & RDS VSD & PDA & CHF	30	F	1390	1400	3	3	6
No. 6	Midgut volvulus	36	M	2790	2850	7	3	8
No. 7	Prematurity & RDS Maternal Amnionitis and Sepsis	28	M	1400	1850	8	4	6
No. 8	Prematurity and RDS, NEC	27	F	1120	1200	17	1	6
No. 9	Prematurity and RDS NEC	27	F	1120	1240	18	1	6
No. 10	Birth Asphyxia, Gastroschisis	38	F	2180	2360	7	2	7
No. 11	Severe Asphyxia, Seizure & NEC	38	M	2620	2650	7	1	3
No. 12	Triplet & RDS, Birth Asphyxia	30	M	960	900	7	0	0
No. 13	Hirschsprung's disease	39	M	3180	3050	8	8	9

CHF = Congestive Heart Failure
IUGR = Intrauterine Growth Retardation
NEC = Necrotizing Enterocolitis
PDA = Patent Ductus Arteriosus
VSD = Ventricular Septal Defect

Table IV

CLINICAL DATA - GROUP II (2 weeks TPN)

PATIENTS	DIAGNOSIS	Gestational Age (wks)	Sex	Birth Weight (g)	Weight at Sampling (g)	Age at Sampling (days)	Apgar Score	
							1 min.	5 min.
No. 1	Prematurity and Sepsis NEC	35	F	1950	2300	18	4	8
No. 2	Prematurity and NEC	30	F	1160	1250	21	2	7
No. 3	Prematurity and IUGR RDS & NEC	37	M	1860	2450	28	4	7
No. 4	IUGR and Birth Asphyxia	36	M	1800	1900	17	0	3
No. 5	NEC and Sepsis	36	F	2650	2700	22	4	8
No. 6	CHF and Sepsis and NEC Coarctation of Aorta	40	F	2500	2860	28	10	10
No. 7	Prematurity & RDS & NEC	30	M	1420	1950	34	3	8
No. 8	Prematurity & RDS & NEC	26	M	930	1150	27	7	8

CHF = Congestive Heart Failure
IUGR = Intrauterine Growth Retardation
NEC = Necrotizing Enterocolitis
RDS = Respiratory Distress Syndrome

Table V

CLINICAL DATA - GROUP III (3 - 8 weeks TPN)

PATIENTS	DIAGNOSIS	Gestational Age (wks)	Sex	Birth Weight (g)	Weight at Sampling (g)	Age at Sampling (days)	Apgar Score	
							1 min.	5 min.
No. 1	Prematurity and Midgut Volvulus Duodenal Stricture	32	M	1980	2700	55	7	8
No. 2	Spina Bifida & Meningomyelocele Hydrocephalus & Sepsis	41	F	2860	4900	78	6	8
No. 3	Prematurity and RDS NEC → Perforated Bowel	27	F	960	1300	35	2	6
No. 4	Prematurity and Sepsis NEC → Perforated Colon	26	M	700	1700	50	4	7
No. 5	Prematurity and Sepsis NEC & Birth Asphyxia	34	M	2190	2900	34	1	4
No. 6	IUGR and Aspiration NEC	35	F	1140	2050	28	9	10
No. 7	Prematurity and RDS NEC → Perforated Colon	29	F	1090	2200	55	3	8
No. 8	Anomalous Pulmonary Veins Imperforate Anus & Midgut Volvulus	40	M	3360	3510	41	7	8
No. 9	Prematurity & VSD & NEC Mesenteric Cyst → Bowel Resection	31	F	2140	2600	70	4	4

IUGR = Intrauterine Growth Retardation
NEC = Necrotizing Enterocolitis
RDS = Respiratory Distress Syndrome
VSD = Ventricular Septal Defect

Table VI

BILE SALT ANALYSIS PROCEDURE: ASSAY

	Blank (ml)	Test (ml)	CDCA Standards (ml)		
Buffer	2.0	2.0	2.0	2.0	2.0
Hydrazine	1.0	1.0	1.0	1.0	1.0
NAD	0.5	0.5	0.5	0.5	0.5
Bile sample	0.003	0.003	-	-	-
Enzyme	-	0.025	0.025	0.025	0.025
Buffer	0.025	-	-	-	-
Chenodeoxycholate	-	-	0.01	0.025	0.05

Table VII

NEONATAL JAUNDICE

	Group I	Group II	Group III
Jaundice	10	5	3
No Jaundice	3	3	6
TOTAL	13	8	9

Table VIII

GROUP I (CONTROL) = Laboratory Data

PATIENTS	CHOLESTEROL m mol/L	%	PHOSPHOLIPID m mol/L	%	BILE ACIDS m mol/L	%
No. 1	0.17	7.7	0.33	15.0	1.7	77.3
2	0.31	10.3	0.63	20.9	2.08	68.9
3	0.02	2.3	0.39	45.9	0.44	51.8
4	0.66	13.4	1.81	36.9	2.44	49.7
5	0.01	2.6	0.152	39.5	0.22	57.9
6	0.21	15.7	0.205	14.9	0.93	69.4
7	0.31	15.2	0.81	39.7	0.92	45.1
8	0.28	14.6	0.70	36.5	0.94	48.9
9	0.39	18.1	0.88	40.7	0.89	41.2
10	0.27	26.1	0.125	12.1	0.64	61.8
11	0.36	24.7	0.137	9.4	0.96	65.9
12	0.46	15.2	1.84	60.9	0.72	23.8
13	0.39	18.2	1.09	50.9	0.66	30.8
Mean ± SEM		14.2±2.0		32.56±4.55		53.27±4.35

Table IX

GROUP II (2 weeks TPN)

PATIENTS	CHOLESTEROL m mol/L	%	PHOSPHOLIPID m mol/L	%	BILE ACIDS m mol/L	%
No. 1	0.41	13.3	0.85	27.6	1.82	59.1
2	0.18	8.5	0.24	11.3	1.7	80.2
3	0.28	11.8	0.49	20.7	1.60	67.5
4	0.39	7.1	0.96	17.6	4.1	75.2
5	0.37	21.5	0.35	20.3	1.0	58.1
6	0.60	8.9	0.93	13.8	5.2	77.3
7	0.48	5.9	0.81	10.0	6.82	84.1
8	0.49	5.8	0.76	9.8	7.12	85.1
Mean ± SEM		10.4±1.9		16.39±2.23		73.33±3.75

Table X

GROUP III (3 - 8 weeks TPN) = Laboratory Data

PATIENTS	CHOLESTEROL		PHOSPHOLIPID		BILE ACIDS	
	m mol/L	%	m mol/L	%	m mol/L	%
No. 1	0.23	13.3	0.54	31.2	0.96	55.5
2	0.43	22.5	0.41	20.5	1.16	58.0
3	0.07	10.3	0.35	51.5	0.26	38.2
4	0.12	23.1	0.24	46.2	0.16	30.8
5	0.36	18.9	0.93	48.9	0.61	32.1
6	0.28	25.9	0.49	35.2	0.54	38.8
7	0.19	16.4	0.61	52.6	0.36	31.0
8	0.26	17.1	1.0	65.8	0.26	17.1
9	0.13	16.2	0.5	62.5	0.17	21.2
Mean ± SEM		18.2±1.9		46.04±4.91		35.86±4.6

Table XI

TOTAL BILIARY LIPID CONCENTRATION (g/dL)

GROUP I		GROUP II		GROUP III	
PATIENT		PATIENT		PATIENT	
1	0.116	1	0.171	1	0.098
2	0.163	2	0.109	2	0.106
3	0.0528	3	0.128	3	0.043
4	0.285	4	0.290	4	0.032
5	0.023	5	0.090	5	0.116
6	0.070	6	0.350	6	0.076
7	0.120	7	0.417	7	0.072
8	0.111	8	0.428	8	0.101
9	0.127			9	0.052
10	0.051				
11	0.072				
12	0.196				
13	0.131				
Mean $\bar{x}$	0.117		0.2478		0.0773
SEM	0.0193		0.0493		0.0100

Figure I

RELATIONSHIP BETWEEN BILIARY CHOLESTEROL
AND DURATION OF THE FASTING PERIOD (Control Group)

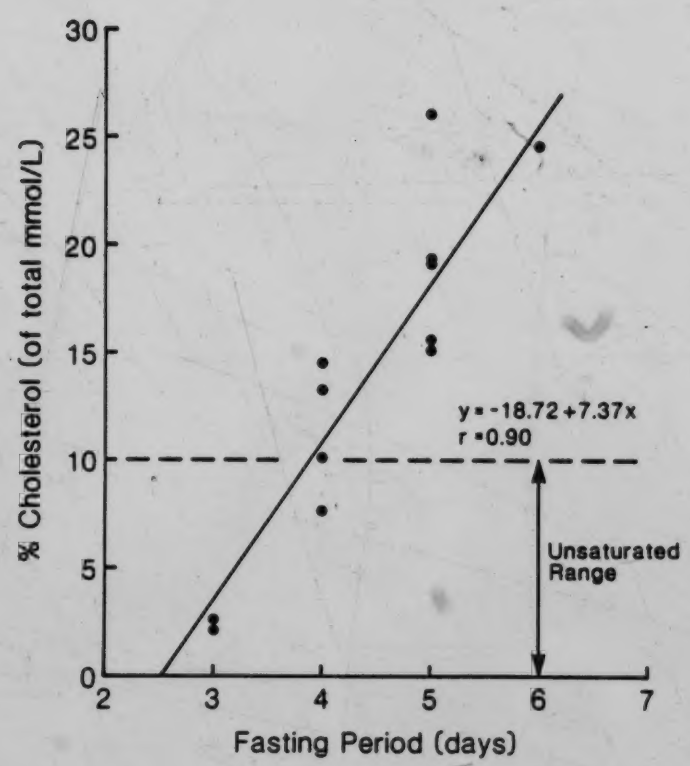


Figure II

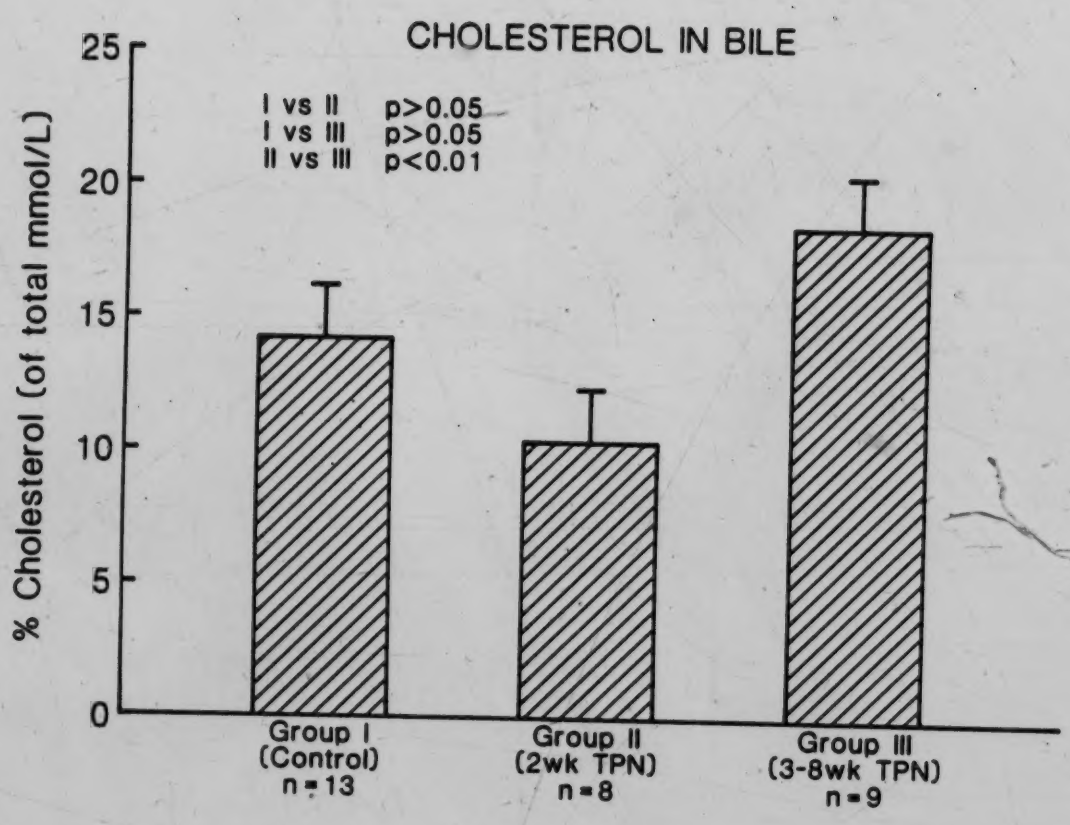

CHOLESTEROL IN BILE
I vs II p>0.05
I vs III p>0.05
II vs III p<0.01
% Cholesterol (of total mmol/L)
25
20
15
10
5
0
Group I
(Control)
n=13
Group II
(2wk TPN)
n=8
Group III
(3-8wk TPN)
n=9

Figure III

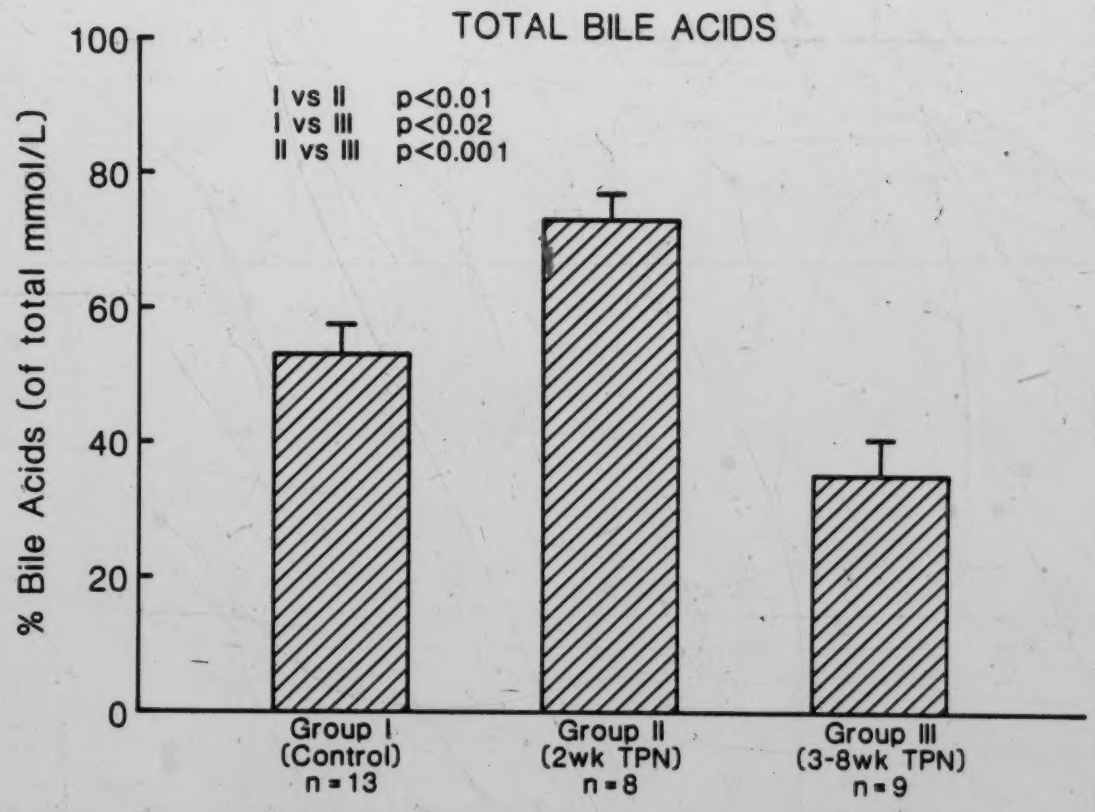

TOTAL BILE ACIDS
I vs II p<0.01
I vs III p<0.02
II vs III p<0.001
% Bile Acids (of total mmol/L)
100
80
60
40
20
0
Group I
(Control)
n=13
Group II
(2wk TPN)
n=8
Group III
(3-8wk TPN)
n=9

Figure IV

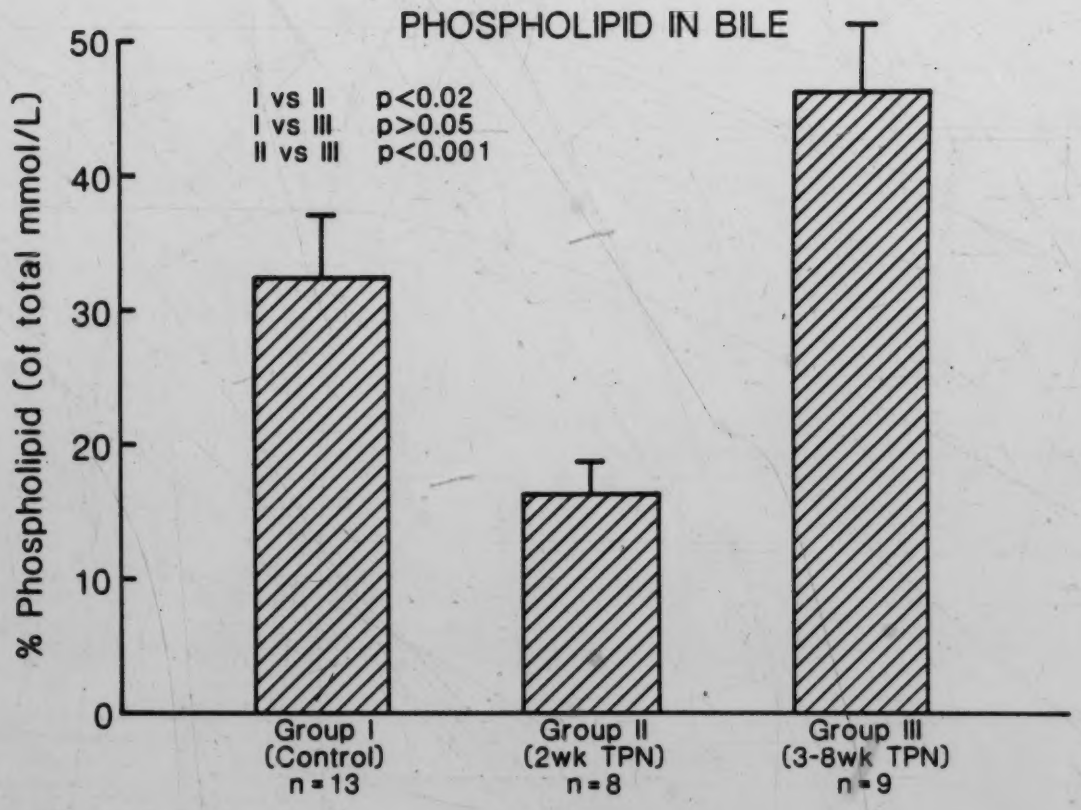

PHOSPHOLIPID IN BILE
I vs II p<0.02
I vs III p>0.05
II vs III p<0.001
% Phospholipid (of total mmol/L)
50
40
30
20
10
0
Group I
(Control)
n=13
Group II
(2wk TPN)
n=8
Group III
(3-8wk TPN)
n=9

Figure V

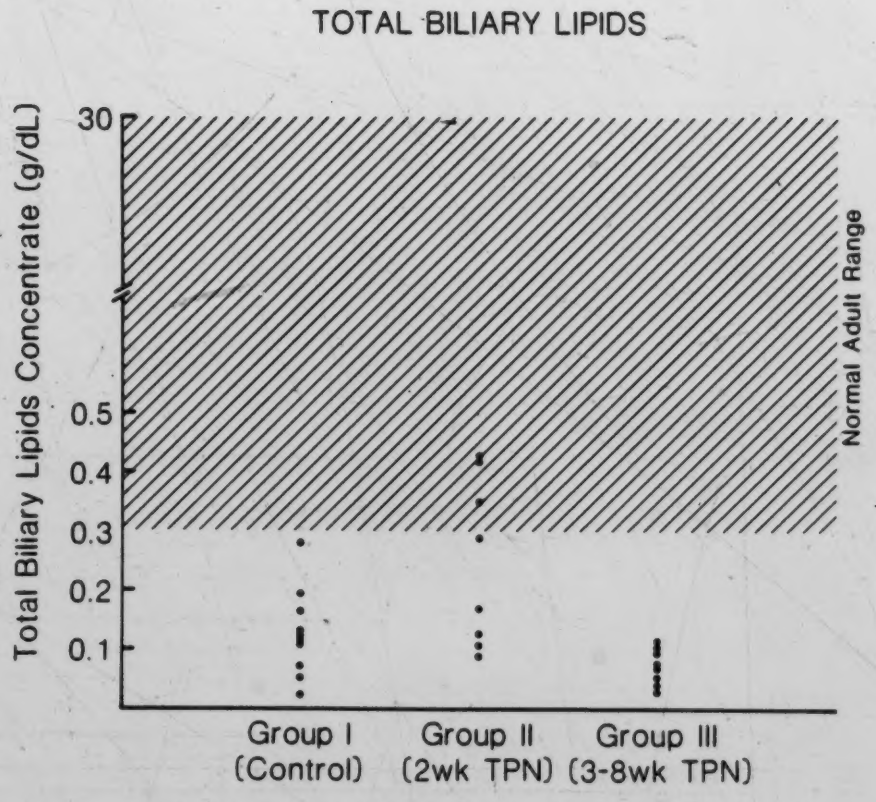

TOTAL BILIARY LIPIDS
Total Biliary Lipids Concentrate (g/dL)
30
0.5
0.4
0.3
0.2
0.1
Normal Adult Range
Group I (Control)
Group II (2wk TPN)
Group III (3-8wk TPN)

Figure VI

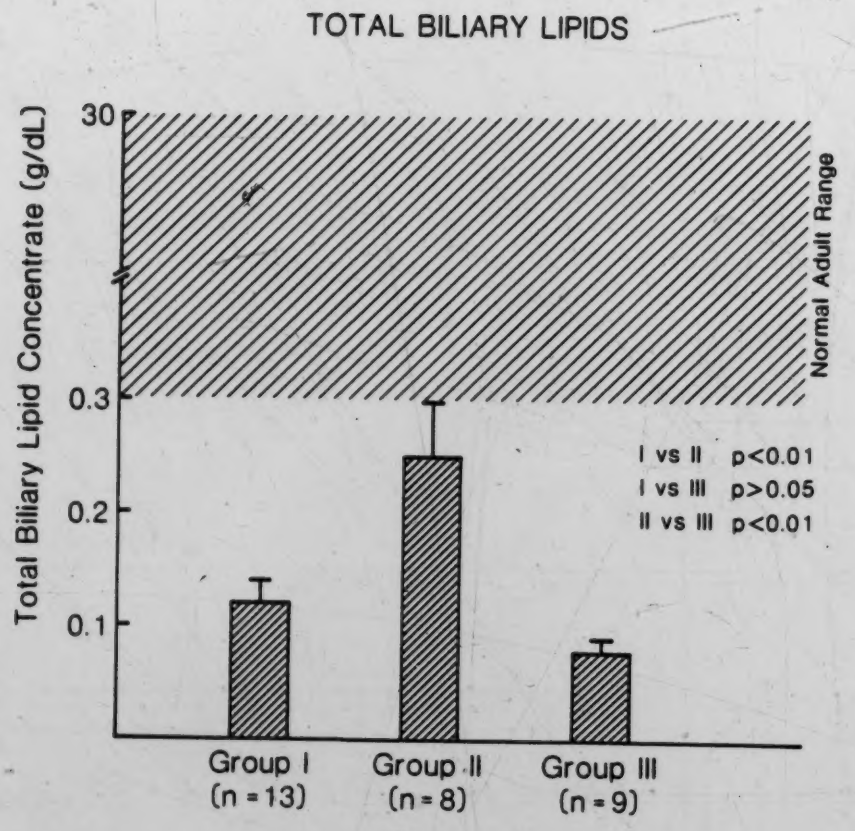

TOTAL BILIARY LIPIDS
Total Biliary Lipid Concentrate (g/dL)
30
0.3
0.2
0.1
Normal Adult Range
I vs II p<0.01
I vs III p>0.05
II vs III p<0.01
Group I
(n = 13)
Group II
(n = 8)
Group III
(n = 9)

Figure VII

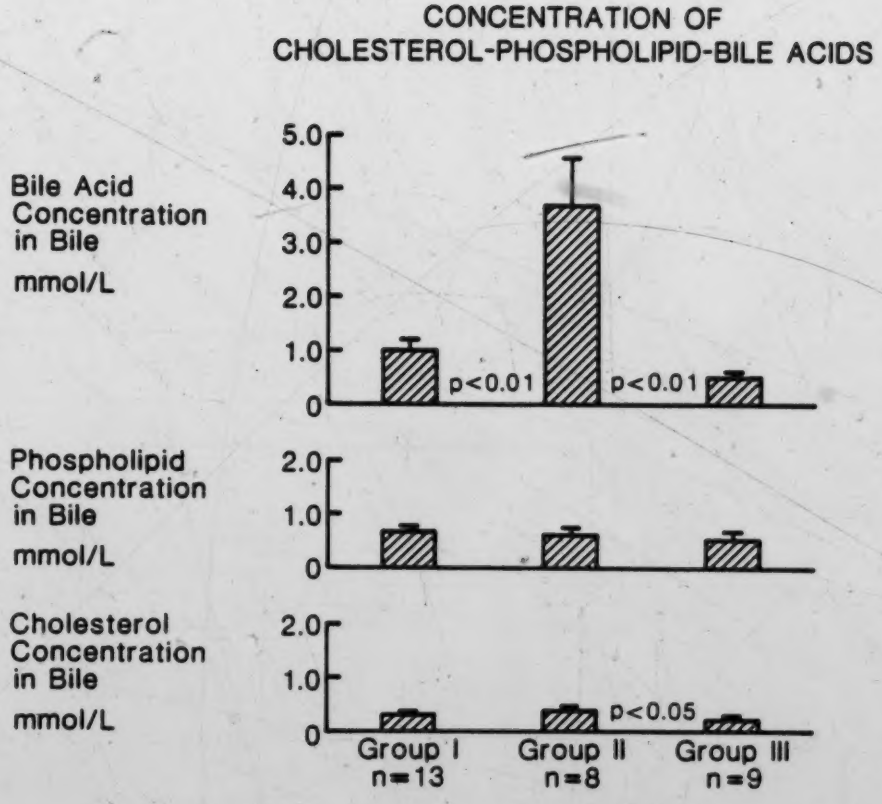

CONCENTRATION OF
CHOLESTEROL-PHOSPHOLIPID-BILE ACIDS
Bile Acid Concentration in Bile mmol/L
5.0
4.0
3.0
2.0
1.0
0
p<0.01
p<0.01
Phospholipid Concentration in Bile mmol/L
2.0
1.0
0
Cholesterol Concentration in Bile mmol/L
2.0
1.0
0
p<0.05
Group I n=13
Group II n=8
Group III n=9

Figure VIII

Critical Micellar Concentration of Bile Acids

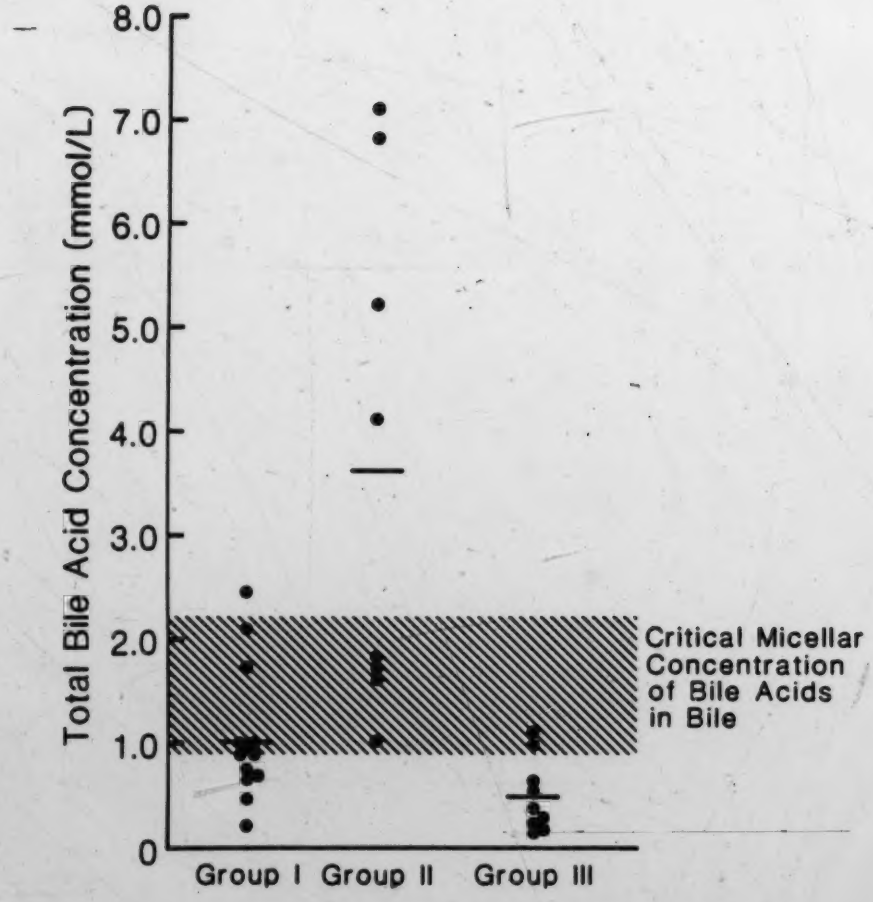
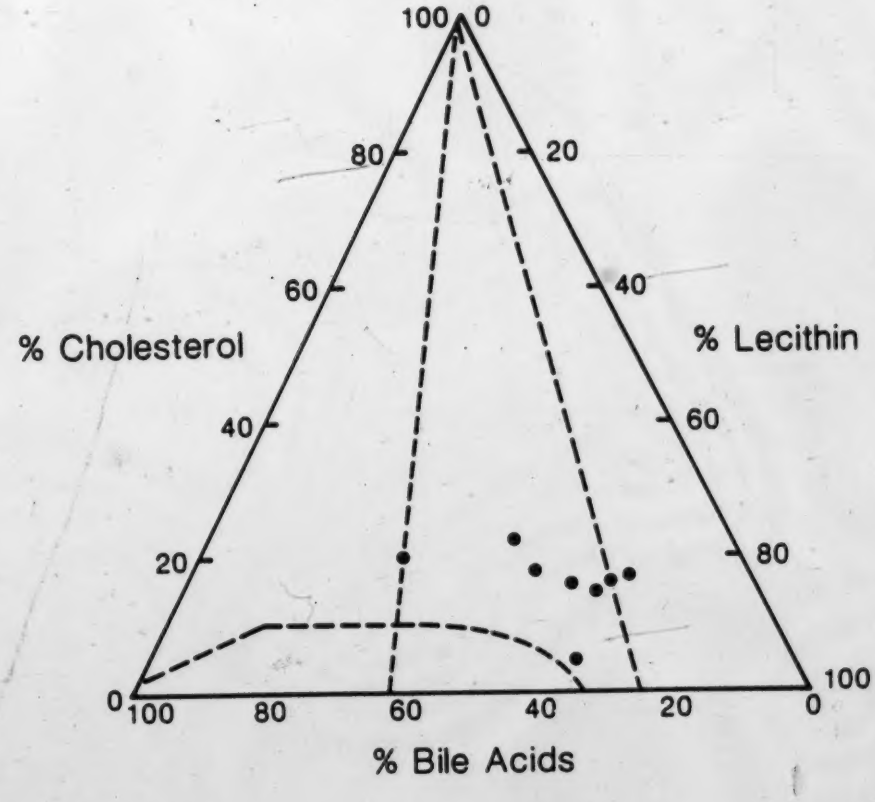


Figure IX

The Admirand and Small Triangular Coordinate



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VITA

NAME: Abdollah A. M. ALrabeeah

DATE OF BIRTH: November 11, 1954

NATIONALITY: Saudi Arabian

HOME ADDRESS: Apartment #507, 11020 - 53 Avenue
Edmonton, Alberta
Canada T6H 0S4

PERMANENT ADDRESS: P.O. Box 54385
Riyadh 11514
Saudi Arabia

PROFESSIONAL QUALIFICATION: July 1979 MBBS(MD)

POST-QUALIFICATION APPOINTMENTS:

Rotating Internship - Sept. 1979 to August 1980
University of Riyadh (King Saud) Hospitals
King Faisal Specialist Hospital

Resident in Surgery:
Sept. 1980 to July 1981
University of Riyadh Hospitals

Joined University of Alberta residency program in General Surgery
October 1981 to June 30, 1986

Research Fellow July 1, 1984 to June 30, 1985